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(54) **DNA MOLECULE ENCODING A VARIANT α_{2B}-ADRENOCEPTOR PROTEIN, AND USES THEREOF**

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(56) **References Cited**

U.S. PATENT DOCUMENTS

5,053,337	A		10/1991	Weinshank et al.
5,350,836	A	*	9/1994	Kopchick et al. 530/399
5,595,880	A		1/1997	Weinshank et al.
5,861,309	A		1/1999	Bard et al.

OTHER PUBLICATIONS

Snapir, A., et al, 2001, J. Am. Coll. Cardiol., 37(6): 1516–1522.*

Ji, et al, 1998, J. Biol. Chem, 273: 17299–17302.*

Baldwin, C.T. et al., "Identification of a Polymorphic Glutamic Acid Stretch in the α_{2B}–Adrenergic Receptor and Lack of Linkage with Essential Hypertension," *Am. J. Hyper.* 12:853–857 (1999).

Baumgart, D. et al., "Augmented α–Adrenergic Constriction of Atherosclerotic Human Coronary Arteries," *Circulation* 99:2090–2097 (1999).

Heinonen, P. et al., "Identification of a three amino acid deletion in the alpha–2B–adrenergic receptor which is associated with reduced basal metabolic rate in obese subjects," *J. Clin. Endocrinol. Metab.* 84:2429–2433 (1999).

Jewell–Motz, E. et al., "An acidic motif within the third intracellular loop of the alpha2C2 adrenergic receptor is required for agonist–promoted phosphorylation and desensitization," *Biochem.* 34:11946–11953 (1995).

Julius, B.K. et al. (1999). "Alpha–adrenoceptor blockade prevents exercise–induce vasoconstriction of stenotic coronary arteries." *J. Am. Coll. Cardiology* 33:1499–1505.

Huchet, A.M., "Central α_1–adrenoceptors and cardiovascular control in normotensive and spontaneously hypertensive rats," *Eur J Pharmacol,* 1983, 95:207–213.

Davey, M., "Mechanism of alpha blockade for blood pressure control," *Am J Cardiol,* 1987, 59:18G–28G.

Leech, C.J. and Faber, J.E., "Different α–adrenoceptor subtypes mediate constriction of arterioles and venules," *Am J Physiol* 270 (*Heart and Circulatory Physiol* 39), 1996, :H710–H722.

Limberger, N. et al., "Pharmacological characterization of presynaptic α_2–autoreceptors in rat submaxillary gland and heart atrium," *Br J Pharmacol,* 1992, 107:246–255.

Bockman, C.S. et al., "Binding and functional characterization of alpha–2 adrenergic receptor subtypes on pig vascular endothelium," *J Pharmacol Exp Therpeut,* 1993, 267:1126–1133.

Reid, J.L., "Alpha–adrenergic receptors and blood pressure control," *Am J Cardiol,* 1986, 57:6E–12E.

Ruffolo, Jr., R.R. et al., "Pharmacologic and Therapeutic applications of α_2–adrenoceptor subtypes," *Annu Rev Pharmacol Toxicol,* 1993, 32:243–279.

Collins, P and Sheridan, D., "Improvement angina pectoris wiht alpha adrenoceptor blockade," *Br Heart J,* 1985, 53:488–492.

Bylund, D.B., "Subtypes of α_1– and α_2–adrenergic receptors," *FASEB J,* 1992, 6:832–839.

(List continued on next page.)

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(57) **ABSTRACT**

This invention relates to a DNA sequence comprising a nucleotide sequence encoding a variant α_{2B}-adrenoceptor protein and to the variant α_{2B}-adrenoceptor protein as well as a method for screening a subject to determine if the subject is a carrier of a variant gene that encodes the variant α_{2B}-adrenoceptor protein. Further this invention relates to a method for treating a mammal suffering from vascular contraction of coronary arteries, the method comprising the step of administering a selective α_{2B}-adrenoceptor antagonist to the mammal and to transgenic animals comprising a human DNA molecule encoding human α_{2B}-adrenoceptor protein or the variant α_{2B}-adrenoceptor protein.

6 Claims, No Drawings

OTHER PUBLICATIONS

Snapir, A. et al., "An insertion/deletion polymorphism in the α_{2B}–adrenergic receptor gene is a novel genetic risk factor for acute coronary events," *J Am Coll Card,* 2001, 37:1516–1522.

Phillips, J.K. et al., "Receptors involved in nerve–mediated vasocontriction in small arteries of the rat hepatic mesentery," *Br J Pharmacol,* 1998, 124:1403–1412.

Michel, A.D. et al., "Assessment of imiloxan as a selective α_{2B}–adrenoceptor antagonist," *Br J Pharmacol,* 1990, 99:560–564.

Link, R.E. et al., "Cardiovascular regulation in mice lacking α_2–adrenergic receptor subtypes b and c," *Science,* 1996, 273:803–805.

MacMillan, L.B. et al., "Central hypotensive effects of the α_{2a}–adrenergic receptor subtype," *Science,* 1996, 273:801–803.

Richman, J.G. and Regan, J.W., "α_2–Adrenergic receptors increase cell migration and decrease F–actin labeling in rat aortic smooth muscle cells," *Am J Physiol,* 1998, 274 (*Cell Physiol* 43):C654–C662.

Wilber, D.J. et al., "α–Adrenergic infulences in canine ischemic sudden death: effects of α_1–adrenoceptor blockade with prazosin," *J Cardiovasc Pharmacol,* 1987, 10:96–106.

Indolfi, C. et al., "Role of α_2–adrenoceptoes in normal and atherosclerotic human coronary circulation," *Circulation,* 1992, 86:1116–1124.

Handy, D.E. et al., "Diverse tissue expression of rat α_2–adrenergic receptor genes," *Hypertension,* 1993, 21:861–865.

Hodgson, J.M. et al., Effects of regional α– and β–blockade on resting and hyperemic coronary blood flow in conscious, unstressed humans, *Circulation,* 1989, 79:797–809.

Redfern, W.S. et al., "The role of α_2–adrenoreceptors in the vasculature of the rat tail," *Br J Pharmacol,* 1995, 114:1724–1730.

Tavares, A. et al., "Localization of α_{2A}– and α_{2B}–adrenergic receptor subtypes in brain," *Hypertension,* 1996, 27:449–455.

* cited by examiner

DNA MOLECULE ENCODING A VARIANT α_{2B}-ADRENOCEPTOR PROTEIN, AND USES THEREOF

This application is a divisional of application Ser. No. 09/422,985 filed Oct. 22, 1999, which is incorporated herein by reference.

FIELD OF THE INVENTION

This invention relates to a DNA molecule encoding a variant human α_{2B}-adrenoceptor, said variant α_{2B}-adrenoceptor protein and a method to assess the risk of individuals to suffer from vascular contraction of coronary arteries in mammals as well as a method for the treatment of vascular contraction of coronary arteries. This invention also relates to transgenic animals comprising a human DNA molecule encoding human α_{2B}-adrenoceptor or said variant α_{2B}-adrenoceptor.

BACKGROUND OF THE INVENTION

The publications and other materials used herein to illuminate the background of the invention, and in particular, cases to provide additional details respecting the practice, are incorporated by reference.

The α_2-adrenoceptors α_2-ARs) mediate many of the physiological effects of the catecholamines norepinephrine and epinephrine. Three genetic subtypes of α_2-adrenoceptors are known in humans and other mammals, denoted as α_{2A}-, α_{2B}-, and α_{2C}-adrenoceptors. The human genes encoding the receptors are located on chromosomes 10, 2 and 4, respectively. No splice variants are known to exist of these receptors, as the genes are intronless. The tissue distributions and physiological and pharmacological functions of the receptor subtypes have been reviewed e.g. by MacDonald et al. (1997) and Docherty (1998). Based on recent studies with gene-targeted and transgenic mice, α_{2A}-adrenoceptors mediate most of the pharmacological actions ascribed to currently available α_2-adrenoceptor agonists, including inhibition of neurotransmitter release, central hypotensive and bradycardic effects, sedation and anesthesia, and analgesia. The same studies indicate that α_{2B}-adrenoceptors mediate peripheral vasoconstriction in response to agonist activation (Link et al. 1996, Macmillan et al. 1996). Other physiological or pharmacological effects have not been associated with certainty with this receptor subtype. The α_{2C}-adrenoceptor subtype appears to be involved in regulation of complex behaviors. It is not known that this subtype would have important functions in peripheral tissues outside the central nervous system or in cardiovascular regulation.

Coronary heart disease (CHD), like many other common disorders, arises from complex interactions between genetic and environmental factors. It is reasonable to assume that functionally important genetic variation in mechanisms important for the regulation of vascular functions, including the coronary vasculature, will be found to be associated with the pathogenesis and therapy of CHD. A variant form of the human α_{2B}-AR gene was recently identified (Heinonen et al., 1999). The variant allele encodes a receptor protein with a deletion of three glutamate residues in an acidic stretch of 18 amino acids (of which 15 are glutamates) located in the third intracellular loop of the receptor polypeptide. This acidic stretch is a unique feature in the primary structure of α_{2B}-AR in comparison to α_{2A}-AR and α_{2C}-AR, suggesting that the motif has a distinct role in the function of α_{2B}-AR. Amino acid sequence alignment of α_{2B}-AR polypeptides of

different mammals reveals that the acidic stretch is highly conserved among the α_{2B}-ARs of mammals and that the acidic stretch is long in humans in comparison to other species. This suggests that the motif is important for the functionality of the receptor, and that the short form (D for "deletion") probably represents the ancestral form and the long form (I for "insertion") could well represent a more recent allelic variant in humans. Jewell-Motz and Liggett (1995) studied the in vitro functions of this stretch using site-directed mutagenesis to delete as well as to substitute 16 amino acids of the stretch. Their results suggest that this acidic motif is necessary for full agonist-promoted receptor phosphorylation and desensitization.

Based on the vasoconstrictive property of α_{2B}-AR in mice and the involvement of this acidic region in the desensitization mechanism of the receptor, we hypothesized that the deletion variant confers reduced receptor desensitization and therefore augmented vasoconstriction that could be associated with cardiovascular pathologies. To test this hypothesis, we carried out a 4-year prospective study in 912 middle-aged Finnish men.

OBJECT AND SUMMARY OF THE INVENTION

One object of this invention is to provide a DNA sequence of a variant human α_{2B}-adrenoceptor gene and the corresponding variant α_{2B}-adrenoceptor protein.

Another object of the invention is to provide a method for screening a subject to assess if an individual is at risk to suffer from vascular contraction of coronary arteries.

A third object of the invention is to provide a method for the treatment of vascular contraction of coronary arteries of mammals.

A fourth object of the invention is to provide a transgenic animal with a gene encoding a human α_{2B}-adrenoceptor or said variant thereof.

Thus, according to one aspect the invention concerns a DNA sequence comprising a nucleotide sequence encoding a variant α_{2B}-adrenoceptor protein with a deletion of at least 1 glutamate from a glutamic acid repeat element of 12 glutamates, amino acids 298–309, in an acidic stretch of 18 amino acids 294–311, located in the 3rd intracellular loop of the receptor polypeptide.

The invention further concerns a variant α_{2B}-adrenoceptor protein with a deletion of at least 1 glutamate from a glutamic acid repeat element of 12 glutamates, amino acids 298–309, in an acidic stretch of 18 amino acids 294–311, located in the 3rd intracellular loop of the receptor polypeptide.

According to another aspect the invention concerns a method for screening a subject to determine if said subject is a carrier of a said variant gene with both alleles encoding a said variant α_{2B}-adrenoceptor, i.e. to determine if said subject's genotype of the human α_{2B}-adrenoceptor is of the deletion/deletion (D/D) type, comprising the steps of

a) providing a biological sample of the subject to be screened,

b) providing an assay for detecting in the biological sample the presence of

   i) the insertion/insertion (I/I) or deletion/insertion (D/I) genotypes of the human α_{2B}-adrenoceptor, or

   ii) the D/D genotype of the human α_{2B}-adrenoceptor, and

c) assessing at least one of the two following

   i) an individual's risk to develop a disease involving vascular contraction of coronary arteries, or

   ii) an individual's need for α_{2B}-selective or α_{2B}-nonselective α_2-adrenoceptor antagonist therapy,

based on whether said subject is of said D/D genotype or not.

According to a third aspect the present invention concerns a method for treating a mammal suffering from vascular contraction of coronary arteries, said method comprising the step of administering a selective α_{2B}-adrenoceptor antagonist to said mammal.

According to a fourth aspect the present invention concerns a transgenic animal which carries a human DNA sequence comprising a nucleotide sequence encoding a human α_{2B}-adrenoceptor protein or a variant thereof.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a DNA molecule encoding a variant human α_{2B}-adrenoceptor, said variant α_{2B}-adrenoceptor protein and a method to assess the risk of individuals to suffer from vascular contraction of coronary arteries in mammals as well as a method for the treatment of vascular contraction of coronary arteries. The present invention also relates to transgenic animals comprising a human DNA molecule encoding a human α_{2B}-adrenoceptor or said variant α_{2B}-adrenoceptor protein.

The word treating shall also be understood to include preventing.

The concept "a deletion of at least 1 glutamate from a glutamic acid repeat element of 12 glutamates" refers to any deletion of 1 to 12 glutamates irrespective of the specific location in, or how many glutamates from said repeat element of 12 glutamates, amino acids 298–309 (SEQ ID NO: 4), in an acidic stretch of 18 amino acids 294–311 located in the 3^{rd} intracellular loop of the receptor polypeptide are deleted.

The concept "deletion/deletion (D/D) genotype of the human α_{2B}-adrenoceptor", in short "D/D genotype", refers to a genotype of an individual having both α_{2B}-adrenoceptor alleles code for a variant α_{2B}-adrenoceptor with a deletion of at least 1 glutamate from a glutamic acid repeat element of 12 glutamates, amino acids 298–309, in an acidic stretch of 18 amino acids 294–311 (SEQ ID NO: 4), located in the 3^{rd} intracellular loop of the receptor polypeptide. Correspondingly "deletion/insertion (D/I) genotype" refers to a genotype having one of the gene alleles code for an α_{2B}-adrenoceptor with a said deletion and the other without a said deletion, i.e. with a respective insertion, and thus the "insertion/insertion (I/I) genotype" refers to a genotype having both alleles code for an α_{2B}-adrenoceptor without said deletion or deletions.

We recently identified a common variant form (SEQ ID NO: 1) of the human α_{2B}-AR gene (SEQ ID NO: 3). This variant gene encodes a receptor protein (SEQ ID NO: 2) with a deletion of 3 glutamates, amino acids 307–309, from a glutamic acid (Glu) repeat element of 12 glutamates, amino acids 298–309, in an acidic stretch of 18 amino acids 294–311 (SEQ ID NO: 4), located in the 3^{rd} intracellular loop of the receptor polypeptide. This variant gene (SEQ ID NO: 1) was associated with decreased basal metabolic rate (BMR) in a group of obese Finnish subjects (Heinonen et al. 1999). Of the 166 obese subjects, 47 (28%) were homozygous for the long 12 glutamate repeat element (Glu^{12}/Glu^{12}), whereas 90 (54%) were heterozygous (Glu^{12}/Glu^{9}) and 29 (17%) were homozygous for the short form (Glu^{9}/Glu^{9}).

The results to be presented below show that in a population-based cohort of 912 Finnish middle-aged men subjects homozygous for the short form (Glu^{9}/Glu^{9}) described above, thus representing a deletion/deletion (D/D) genotype of the α_{2B}-adrenoceptor, have a significantly elevated risk for acute coronary events in a four-year follow-up study. The risk for an acute coronary event, defined as definite or possible acute myocardial infarction (AMI) or prolonged (>20 min) chest pain requiring hospitalization, was increased 2.5 fold in subjects who had this D/D genotype. This increase in the risk for acute coronary events is as great as so far observed for any other genetic risk factor for acute coronary events or acute myocardial infarction in a prospective population study. Also the frequency of a study subject having a history of coronary heart disease (CHD) as well as CHD in an exercise test was associated with this D/D genotype. Based on these results and previous publications referred to above it can be postulated that this D/D genotype is related to an impaired capacity to downregulate α_{2B}-adrenoceptor function during sustained receptor activation. Since altered α_{2B}-adrenoceptor function seems to be of relevance in the pathogenesis of a significant fraction of all cases of acute coronary events in subjects with this D/D genotype (homozygous Glu^{9}/Glu^{9}) we believe it could also be of relevance in subjects with the insertion/deletion (I/D) (heterozygous Glu^{12}/Glu^{9}) and insertion/insertion (I/I) (homozygous Glu^{12}/Glu^{12}) genotypes when other risk factors for AMI are present. Further, since this specific deletion of 3 glutamates, amino acids 307–309, from said glutamic acid repeat element of 12 glutamates, amino acids 298–309, in said acidic stretch of 18 amino acids 294–311, located in the 3^{rd} intracellular loop of the receptor polypeptide seems to be of relevance in cases of AMI we believe that also other deletions, i.e. deletions of at least 1 glutamate, from said glutamic acid repeat element of 12 glutamates, amino acids 298–309, could be of relevance in the pathogenesis of AMI, because the 3^{rd} intracellular loop of the receptor polypeptide it is located in seems to have an essential role in the downregulation of the α_{2B}-adrenoceptor.

Thus based on the results to be presented below and the publications referred to above an α_{2B}-adrenoceptor antagonist would be useful for treating a mammal suffering from vascular contraction of coronary arteries.

Furthermore, an α_{2B}-adrenoceptor antagonist selective for the α_{2B}-adrenoceptor subtype would be therapeutically beneficial for the treatment of a disease involving said vascular contraction of coronary arteries. Such a disease could be clinically expressed as chronic angina pectoris, specifically e.g. AMI, unstable angina pectoris or Prinzmetal's variant form of angina pectoris. If α_{2B}-adrenoceptor dependent vasoconstriction is a causative factor in some cases of AMI, then antagonism of these receptors should restore coronary circulation and reduce the ischemic myocardial damage. An α_{2B}-adrenoceptor antagonist will relieve the vaso-constrictive component in the sustained ischemic episode of unstable angina pectoris, thus alleviating the symptoms and preventing AMI. Vasoconstriction is a key factor in the pathogenesis of Prinzmetal's angina, and an α_{2B}-adrenoceptor antagonist may resolve and prevent attacks. An α_{2B}-adrenoceptor antagonist will help to alleviate the vasoconstrictive component in all types of CHD, providing both symptomatic relief and protection from AMI.

α_{2B}-adrenoceptors mediate vascular contraction of coronary arteries, and genetic polymorphism present in the α_{2B}-adrenoceptor gene renders some subjects more susceptible to α_{2B}-adrenoceptor mediated vasoconstriction of coronary arteries and associated clinical disorders. These subjects will especially benefit from treatment with an α_{2B}-adrenoceptor antagonist, and will be at increased risk for adverse effects if subtype-nonselective α_2-agonists are administered to them. Therefore, a gene test recognizing subjects with a deletion variant of the α_{2B}-adrenoceptor gene will be useful in diagnostics and patient selection for

specific therapeutic procedures. A gene test recognizing the D/D genotype of the α_{2B}-adrenoceptor is useful in assessing an individual's risk to develop AMI and other clinical disorders involving vascular contraction of coronary arteries related to the D/D genotype. A gene test recognizing the D/D genotype of the α_{2B}-adrenoceptor is useful in selecting drug therapy for patients with diseases involving vascular contraction of coronary arteries associated with the D/D genotype; subjects with the D/D genotype will especially benefit from therapy with α_2-adrenoceptor antagonists α_{2B}-selective or nonselective). A gene test recognizing the D/D genotype of the α_{2B}-adrenoceptor is useful in selecting drug therapy for patients who might be at increased risk for adverse effects of α_2-adrenergic agonists; either, it will be possible to avoid the use of α_2-agonists in such patients, or it will be possible to include a specific α_{2B}-antagonist in their therapeutic regimen.

The DNA sequence can be used for screening a subject to determine if said subject is a carrier of a variant gene. The determination can be carried out either as a DNA analysis according to well known methods, which include direct DNA sequencing of the normal and variant gene, allele specific amplification using the polymerase chain reaction (PCR) enabling detection of either normal or variant sequence, or by indirect detection of the normal or variant gene by various molecular biology methods including e.g. PCR-single stranded conformation polymorphism (SSCP) method or denaturing gradient gel electrophoresis (DGGE). Determination of the normal or variant gene can also be done by using a restriction fragment length polymorphism (RFLP) method, which is particularly suitable for genotyping large numbers of samples. Similarly, a test based on gene chip technology can be easily developed in analogy with many currently existing such tests for single-nucleotide polymorphisms.

The determination can also be carried out at the level of RNA by analyzing RNA expressed at tissue level using various methods. Allele specific probes can be designed for hybridization. Hybridization can be done e.g. using Northern blot, RNase protection assay or in situ hybridization methods. RNA derived from the normal or variant gene can also be analyzed by converting tissue RNA first to cDNA and thereafter amplifying cDNA by an allele specific PCR method.

As examples of useful α_{2B}-adrenoceptor antagonists can be mentioned imiloxan[2-(1-ethyl-2-imidazoyl)methyl-1,4-benzodioxan, ARC-239 [2-[2-(4-(2-methoxy-phenyl) piperazin-1-yl)ethyl]-4,4-dimethyl-1,3-(2H,4H)-isoquinolindione], prazosin[1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-furanylcarbonyl)piperazine] and chlorpromazine[2-chloro-N,N-dimethyl-10H-phenothiazine-10-propanamine].

The required dosage of the compounds will vary with the particular condition being treated, the severity of the condition, the duration of the treatment, the administration route and the specific compound being employed. A typical therapeutically effective daily dose administered, e.g. orally or by infusion, can vary from e.g. 0.1 μg to 10 mg per kilogram body weight of an adult person.

Influence of the variant gene sequence can be investigated in transgenic animals. A transgenic animal can be generated e.g. using targeted homologous recombination methodology. This will provide an ideal preclinical model to investigate and screen new drug molecules, which are designed to modify the influence of the variant gene.

The invention will be described in more detail in the experimental section.

EXPERIMENTAL SECTION

Determination of Genomic Alleles Encoding the α_{2B}-adrenoceptor

PCR-SSCA Analysis

The polymerase chain reaction-single stranded conformational analysis (PCR-SSCA) used to identify the genomic alleles encoding the α_{2B}-adrenoceptor was carried out as follows: The genomic DNA encoding the α_{2B}-adrenergic receptor was amplified in two parts specific for the intronless α_{2B}-adrenoceptor gene sequence (Lomasney et al. 1990). The PCR primer pairs for PCR amplification were as follows: Pair 1: 5'-GGGGCGACGCTCTTGTCTA-3' (SEQ ID NO: 5) and 5'-GGTCTCCCCCTCCTCCTTC-3' (SEQ ID NO: 6) (product size 878 bp), pair 2: 5'-GCAGCAACCGCAGAGGTC-3' (SEQ ID NO: 7) and 5'-GGGCAAGAAGCAGGGTGAC-3' (SEQ ID NO: 8) (product size 814 bp). The primers were delivered by KeboLab (Helsinki, Finland). PCR amplification was conducted in a 5 μl volume containing 100 ng genomic DNA (isolated from whole blood), 2.5 mmol/l of each primer, 1.0 mmol/l deoxy-NTPs, 30 nmol/l ^{33}P-dCTP and 0.25 U AmpliTaq DNA polymerase (Perkin Elmer Cetus, Norwalk, Conn.). PCR conditions were optimized using the PCR Optimizer™ kit (Invitrogen, San Diego, Calif.). Samples were amplified with a GeneAmp PCR System 9600 (Perkin Elmer Cetus). PCR products were digested with restriction enzymes for SSCA analysis. The product of primer pair 1 was digested with Dde I and Dra III (Promega Corp., Madison, Wis.). The product of primer pair 2 was digested with Alu I and Hinc II (Promega Corp.). The digested samples were mixed with SSCA buffer containing 95% formamide, 10 mmol/l NaOH, 0.05% xylene cyanol and 0.05% bromophenol blue (total volume 25 μl). Before loading, the samples were denatured for 5 min at 95° C. and kept 5 min on ice. Three microliters of each sample were loaded on MDE™ high-resolution gel (FMC, BioProducts, Rockland, Mass.). The gel electrophoresis was performed twice, at two different running conditions: 6% MDE gel at +4° C. and 3% MDE gel at room temperature, both at 4 W constant power for 16 h. The gels were dried and autoradiography was performed by apposing to Kodak BioMax MR film for 24 h at room temperature.

Sequencing and Genotyping

DNA samples migrating at different rates in SSCA were sequenced with the Thermo Sequenase™ Cycle Sequencing Kit (Amersham Life Science, Cleveland, Ohio).

For genotyping the identified 3-glutamic acid deletion, DNA was extracted from peripheral blood using standard methods. The α_{2B}-AR I/D genotype was determined by separating PCR-amplified DNA fragments with electrophoresis. Based on the nature of the I/D variant, identification of the long and short alleles was achieved by their different electrophoretic migration rates due to their 9 bp size difference.

The region of interest was amplified using a sense primer 5'-AGGGTGTTTGTGGGGCATCT-3' (SEQ ID NO: 9) and an anti-sense primer 5'-CAAGCTGAGGCCGGA GACACT-3' (SEQ ID NO: 10) (Oligold, Eurogentec, Belgium), yielding a product size of 112 bp for the long allele (I) and 103 bp for the short allele (D). PCR amplification was conducted in a 10 μL volume containing ~100 ng genomic DNA, 1×buffer G (Invitrogen, San Diego, Calif., USA), 0.8 mM dNTPs, 0.3 μM of each primer and 0.25 units of AmpliTaq DNA polymerase (Perkin Elmer Cetus, Norwalk, Conn., USA). Samples were amplified with a GeneAmp PCR System 9600 (Perkin Elmer Cetus). After initial denaturation at 94° C. for 2 minutes, the samples were

amplified over 35 cycles. PCR amplification conditions were 96° C. (40 s), 69° C. (30 s) and 72° C. (30 s) followed by final extension at 72° C. for 6 minutes. The PCR products representing the long and short alleles were identified by two alternative methods.

1) The amplified samples were mixed with 4 μl of stop solution (Thermo Sequenase™ Cycle Sequencing kit), heated to 95° C. for 2 min, and loaded hot onto sequencing gels (Long Ranger™, FMC). The gels were dried and autoradiography was performed as previously described.

2) Separation of the amplified PCR products was performed with electrophoresis on a high-resolution 4% Metaphor agarose gel (FMC Bioproducts, Rockland, Me.) and the bands were visualized by ethidium bromide staining. In both methods, the long (Glu^{12}) and short (Glu^{9}) alleles were identified based on their different electrophoretic migration rates.

Follow-up Study

The above referred four-year follow-up study of 912 Finnish middle-aged men subjects including 192 subjects with a specific deletion/deletion (D/D) genotype of the α_{2B}-adrenoceptor is described in more detail in the following:

Knowing the vasoconstrictive property of α_{2B}-AR in mice and the possible involvement of the investigated acidic region in the desensitization mechanism of the receptor we hypothesized that the observed insertion/deletion allelic variation could be associated with cardiovascular pathologies such as AMI. To test this hypothesis, we carried out a four-year follow-up study in 912 middle-aged Finnish men with no prior history of AMI. The study was carried out as part of the Kuopio Ischemic Heart Disease Risk Factor Study (KIHD), which is an ongoing population-based study designed to investigate risk factors for cardiovascular diseases and related outcomes in men from eastern Finland (Salonen 1988). This area is known for its homogenous population (Sajantila et al. 1996) and high coronary morbidity and mortality rates (Keys 1980).

Of the 912 subjects, 192 (21%) had the D/D genotype, 256 (28%) had the I/I genotype and 464 (51%) were heterozygous i.e. I/D. This genotype distribution is in Hardy-Weinberg equilibrium (p=0.46).

Of the 37 cases that had an acute coronary event during the follow-up, 18 were classified as definite AMI, 12 as possible AMI and seven as prolonged chest pain. Among the subjects with the D/D genotype, 15 (8%) had an acute coronary event during the follow-up time. The corresponding incidences for the I/I and the heterozygous genotypes i.e. I/D were 10 (4%) and 12 (3%). The observed cumulative incidence of acute coronary events differed significantly among the different genotypes (p=0.008). No significant difference in the cumulative incidence of acute coronary events was found between the I/D and the I/I genotypes (p=0.4) (table 1). There was a significant difference (log-rank p=0.0045) between the D/D subgroup and the other two genotypes combined in the cumulative event-free time in the Kaplan-Meier survival function, demonstrating that there is a consistently increased incidence of acute coronary events in the D/D subgroup.

The D/D genotype was associated with a 2.5 fold increased risk for an acute coronary event (95% CI=1.3–4.8, p=0.006) in comparison to the other two genotypes combined. The relative risk remained above 2 after adjustment for major CHD risk factors (table 2).

The D/D subgroup was not significantly different from the I/D+I/I subgroup in terms of many known major risk factors for CHD. From 87 variables in the study database only 5 were significantly different between the D/D and the I/D+I/I genotype subgroups: 1. there were more acute coronary events in the D/D subgroup (8% vs. 3%, p=0.006), 2. history of CHD was more prevalent in the D/D subgroup (37% vs. 29%, p=0.043), 3. the prevalence of CHD in exercise test was higher in the D/D subgroup (30% vs. 22%, p=0.036), 4. mean hemoglobin level was higher in the D/D subgroup (149.0 g/l vs. 146.8 g/l, p=0.005) and 5. mean dietary cholesterol intake (4-days) was lower in the D/D subgroup (411.6 mg vs. 440.1 mg, p=0.033) (table 3). The first four observed differences support our hypothesis that the D/D genotype confers reduced receptor desensitization and therefore augmented vasoconstriction. This augmented vasoconstriction is the reason for the increased incidence of acute coronary events, the higher prevalence of CHD in exercise and history of CHD. We hypothesize that the increased level of hemoglobin is due to relative anoxia of tissues because of this augmented vasoconstriction.

To examine the possibility that the D/D genotype is a genetic marker for acute coronary events rather than a causative factor, we have searched the literature for known genetic risk factors for acute coronary events and AMI and their chromosomal localization. All but one (Apo-B) are on different chromosomes than the α_{2B}-AR gene (chromosome 2) and the gene for Apo-B is neither in the physical nor the genetic vicinity of the α_{2B}-AR gene. Cox regression analysis revealed that the increased RR for acute coronary events in the D/D subgroup is not affected by the serum Apo-B concentration.

Taken together, the known biological properties of the α_{2B}-AR, the homogeneity of the Finnish population with its relatively high incidence of CHD, the study design, the relatively large representative study population and the clustering of the findings around one trait suggest that the D/D receptor allele is a causal genetic risk factor for acute coronary events.

TABLE 1

The cumulative incidence of acute coronary events among men with different genotypes of the α_{2B}-AR (p values are stated below)

Genotype	Events (% of men at risk)	Men at risk (% of all)
D/D	observed	15 (8)	192 (21)
	expected	7.8	
I/D	observed	12 (3)	464 (51)
	expected	18.8	
I/I	observed	10 (4)	256 (28)
	expected	10.4	
I/D + I/I	observed	22 (3)	720 (79)
	expected	29.2	
Total	observed	37 (4)	912 (100)

P values for the above table:

D/D vs. I/D vs. I/I	p = 0.008
D/D vs. I/D	p = 0.002
D/D vs. I/I	p = 0.038
I/D vs. I/I	p = 0.389
D/D vs. I/D + I/I	p = 0.005

TABLE 2

Relative risk (RR) and its 95% confidence interval (CI) for an acute coronary event - a comparison of each of the genotypes with the other two combined. Results of a Cox regression model for 37 acute coronary events in a population sample of 912 subjects

Genotype	Events/men at risk	RR (95% CI) p	Adjusted RR (95% CI) p
D/D	15/192	2.5(1.3–4.8) 0.006	2.3(1.2–4.5) 0.014
I/D	12/464	0.44(0.2–0.9) 0.020	0.5(0.2–1.0) 0.052
I/I	10/256	1.03(0.5–2.1) 0.940	0.96(0.5–2.0) 0.901

Adjustment was done for age, CHD in the family, high cholesterol in the family, hypertension and smoking

TABLE 3

List of all significant differences ($p < 0.05$) between the D/D and the I/D + I/I genotype subgroups among 87 variables in the study database

Variable	D/D	I/D + I/I	p
Acute coronary events [event/n (%)]	15/192 (8)	22/720 (3)	0.006
Ischemic findings in exercise test [case/n (%)]	57/192 (30)	160/720 (22)	0.036
History of CHD [case/n (%)]	71/192 (37)	209/720 (29)	0.043
Mean blood haemoglobin [g/L]	149.0	146.8	0.005
Mean 4 day dietary cholesterol intake [mg]	411.6	440.1	0.033

% = Percent of men at risk

It will be appreciated that the methods of the present invention can be incorporated in the form of a variety of embodiments, only a few of which are disclosed herein. It will be apparent for the specialist in the field that other embodiments exist and do not depart from the spirit of the invention. Thus, the described embodiments are illustrative and should not be construed as restrictive.

References

Docherty J R: Subtypes of functional α_1- and α_2-receptors. *Eur J Pharmacol* 1998;361:1–15

Heinonen P, Koulu M, Pesonen U, Karvonen M, Rissanen A, Laakso M, Valve R, Uusitupa M, Scheinin M: Identification of a three amino acid deletion in the alpha-2B-adrenergic receptor which is associated with reduced basal metabolic rate in obese subjects. *J Clin Endocrinol Metab* 1999;84:2429–2433

Jewell-Motz E, Liggett S B: An acidic motif within the third intracellular loop of the alpha2C2 adrenergic receptor is required for agonist-promoted phosphorylation and desensitization. *Biochemistry* 1995;34:11946–11953

Keys A: *Seven Countries: A Multivariate Analysis of Death and Coronary Heart Disease.* Cambridge, Mass., Harvard University Press, 1980

Link R E, Desai K, Hein L, Stevens M E, Chruscinski A, Bernstein D, Barsh G S, Kobilka B K: Cardiovascular regulation in mice lacking alpha2-adrenergic receptor subtypes b and c. *Science* 1996;273:803–805

Lomasney J W, Lorenz W, Allen L F, King K, Regan J W, Yang-Feng T L, Caron M C, Lefkowitz R J: Expansion of the alpha-2 adrenergic receptor family: cloning and characterization of a human alpha-2 adrenergic receptor subtype, the gene for which is located on chromosome 2. *Proc Natl Acad Sci USA.* 1990;87:5094–5098.

MacDonald E, Kobilka B K, Scheinin M: Gene targeting—homing in on α_2-adrenoceptor subtype function. *Trends Pharmacol Sci* 1997;18:211–219

MacMillan L B, Hein L, Smith M S, Piascik M T, Limbird L E: Central hypotensive effects of the alpha2a-adrenergic receptor subtype. *Science* 1996;273:801–803

Sajantila A, Salem A H, Savolainen P, Bauer K, Gierig C, Paabo S: Paternal and maternal DNA lineages reveal a bottleneck in the founding of the Finnish population. *Proc.Natl.Acad.Sci. U.S.A.* 1996;93:12035–12039

Salonen J T: Is there a continuing need for longitudinal epidemiologic research? The Kuopio Ischaemic Heart Disease Risk Factor Study. *Ann.Clin Res* 1988;20:46–50

SEQUENCE LISTING

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gcg gcc atc acc ttc ctc att ctc ttt acc atc ttc ggc aac gct ctg       96
Ala Ala Ile Thr Phe Leu Ile Leu Phe Thr Ile Phe Gly Asn Ala Leu
             20                  25                  30

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 65                  70                  75                  80

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Arg Arg Thr Trp Cys Glu Val Tyr Leu Ala Leu Asp Val Leu Phe Cys
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Thr Ser Ser Ile Val His Leu Cys Ala Ile Ser Leu Asp Arg Tyr Trp
            100                 105                 110

gcc gtg agc cgc gcg ctg gag tac aac tcc aag cgc acc ccg cgc cgc      384
Ala Val Ser Arg Ala Leu Glu Tyr Asn Ser Lys Arg Thr Pro Arg Arg
        115                 120                 125

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Ile Lys Cys Ile Ile Leu Thr Val Trp Leu Ile Ala Ala Val Ile Ser
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Arg Pro Gln Cys Lys Leu Asn Gln Glu Ala Trp Tyr Ile Leu Ala Ser
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ctg cgc atc tac ctg atc gcc aaa cgc agc aac cgc aga ggt ccc agg      624
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225                 230                 235                 240

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        275                 280                 285

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Cys Gly Ala Ser Pro Glu Asp Glu Ala Glu Glu Glu Glu Glu Glu Glu
    290                 295                 300

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305                 310                 315                 320

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                 85                  90                  95

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            100                 105                 110

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Arg Gly Gln Val Leu Leu Gly Arg Gly Val Gly Ala Ile Gly Gly Gln
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Val Leu Ala Val Val Ile Gly Val Phe Val Leu Cys Trp Phe Pro Phe
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             20                  25                  30

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Leu Phe Leu Val Ser Leu Ala Ala Ala Asp Ile Leu Val Ala Thr Leu
50 55 60

Ile Ile Pro Phe Ser Leu Ala Asn Glu Leu Leu Gly Tyr Trp Tyr Phe
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Arg Arg Thr Trp Cys Glu Val Tyr Leu Ala Leu Asp Val Leu Phe Cys
85 90 95

Thr Ser Ser Ile Val His Leu Cys Ala Ile Ser Leu Asp Arg Tyr Trp
100 105 110

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His Gly Gly Ala Leu Ala Ser Ala Lys Leu Pro Ala Leu Ala Ser Val
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Ala Ser Ala Arg Glu Val Asn Gly His Ser Lys Ser Thr Gly Glu Lys
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385 390 395 400

Cys Lys Val Pro His Gly Leu Phe Gln Phe Phe Phe Trp Ile Gly Tyr
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Cys Asn Ser Ser Leu Asn Pro Val Ile Tyr Thr Ile Phe Asn Gln Asp
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What is claimed is:

1. An isolated DNA sequence comprising a nucleotide sequence encoding a variant α_{2B}-adrenoceptor protein wherein the variation is a deletion of 3 glutamate residues from a glutamic acid repeat element of 12 glutamate residues, amino acids 298–309, in a wildtype α_{2B}-adrenoceptor protein having an amino acid sequence set forth in SEQ ID NO:4.

2. The isolated DNA sequence according to claim **1** comprising the genomic nucleotide sequence of SEQ ID NO:1.

3. The isolated DNA sequence according to claim **1** comprising the amino acid sequence of SEQ ID NO:2.

4. The isolated DNA sequence of according to claim **1** wherein said DNA sequence is cDNA.

5. An isolated RNA sequence comprising an RNA sequence corresponding to the isolated DNA sequence of claim **1**.

6. A hybridizing probe which comprises a single strand of the cDNA according to claim **4**.

* * * * *